

Amendments to the Specification:

Please replace the paragraph beginning at page 84, line 28, with the following redlined paragraph:

Figure 41A depicts a schematic representation of the structure of an anti-murine 4-1BB (CD137) scFv Ig-CD80 fusion protein. **Figure 41B** illustrates cell surface expression of 1D8 (anti-murine 4-1BB) scFv IgG WTH WTCH2CH3-CD80 fusion protein on K1735 melanoma cells by flow immunofluorimetry (~~Fig. 41A~~). The scFv fusion protein was detected with phycoerythrin-conjugated F(ab')₂ goat anti-human IgG. **Fig. 41CB** depicts growth of tumors in naive C3H mice transplanted by subcutaneous injection with wild type K1735 melanoma cells (K1735-WT) or with K1735 cells transfected with 1D8 scFv IgG WTH WTCH2CH3-CD80 (K1735-1D8). Tumor growth was monitored by measuring the size of the tumor. **Fig. 41C-41D** demonstrates the kinetics of tumor growth in naive C3H mice injected intraperitoneally with monoclonal antibodies to remove CD8⁺, CD4⁺, or both CD4⁺ and CD8⁺ T cells prior to transplantation of the animals with K1735-1D8 cells.

Please replace the paragraph beginning at page 86, line 5, with the following redlined paragraph:

Figure 47 shows cell surface expression of an anti-human CD3 scFv IgG WTH WTCH2CH3-CD80 (SEQ ID NO: 443) fusion protein on Reh cells (~~Fig. 47Aupper graph~~) and T51 lymphoblastoid cells (~~Fig. 47Blower graph~~) by measuring the linear fluorescent equivalent (LFE) using flow immunocytofluorimetry.

Please replace the paragraph beginning at page 86, line 9, with the following redlined paragraph:

Figure 48 presents the percent specific killing of untransfected Reh and T51 cells and the percent specific killing of Reh cells (Reh anti-hCD3) (~~Fig. 48Aupper graph~~) and T51 cells (T51 anti-hCD3) (~~Fig. 48Blower graph~~) that were transfected with a construct encoding scFv antibodies specific for human CD3, fused to human IgG1 wild-type hinge-CH2-CH3,

which was fused to human CD80 transmembrane and cytoplasmic tail domains. Human PBMC (effector cells) were combined with BJAB target cells at the ratios indicated.

Please replace the paragraph beginning at page 211, line 16, with the following redlined paragraph:

This example describes construction of an anti-murine 4-1 BB (CD 137) scFv fusion protein that has an IgG wild type hinge and CH2 and CH3 domains that is fused to the CD80 transmembrane and cytoplasmic domains (Fig. 41A). The Example also illustrates the effect of the cell surface expression of the anti-4-1BB scFv IgG CD80 polypeptide when the transfected tumor cells are transplanted into mice.

Please replace the paragraph beginning at page 212, line 9, with the following redlined paragraph:

The retroviral constructs were transfected into the metastatic M2 clone of K1735, a melanoma cell line; provided by Dr. I. Hellstrom, PNRI, Seattle, WA. Transfected cells were screened to select clones that were expressing scFv-Ig fusion proteins on the cell surface. To demonstrate that the 1D8 scFv IgG-CD80 construct was expressed on the cell surface of the tumor cells, the transfected cells were analyzed by flow immunocytometry. Transfected cells (K1735-1D8) were incubated for one hour on ice in phycoerythrin-conjugated F(ab')₂ goat anti-human IgG. The unbound conjugate was then removed by washing the cells and flow cytometry analysis was performed using a Coulter Epics XL cell sorter. Results are presented in Figure 41A 41B.

Please replace the paragraph beginning at page 212, line 19, with the following redlined paragraph:

The growth of K1735-1D8 transfected cells was examined *in vivo*. K1735-WT cells grew progressively when transplanted subcutaneously (s.c.) in naive C3H mice. Although the same dose of K1735-1D8 cells initially formed tumors of an approximately 30 mm² surface

area, the tumors started to regress around day 7 and had disappeared by day 20 as shown in Figure-41B ~~41C~~. Tumor cells that were transfected with a similarly constructed vector encoding a non-binding scFv, a human anti-CD28 scFv construct, grew as well as tumor cells that had not been transfected. The presence of a foreign protein, that is, human IgG1 constant domains or rat variable regions, did not make transfected K1735-WT cells immunogenic; the growth of the K1735-1D8 cells in C3H mice was identical to that of K1735-WT cells (untransfected).

Please replace the paragraph beginning at page 212, line 30, with the following redlined paragraph:

To investigate the roles of CD4⁺ and CD8⁺ T lymphocytes and NK cells in the regression of K1735-1D8 tumors, naive mice were injected intraperitoneally (i.p.) with monoclonal antibodies (monoclonal antibodies, typically 50 µg in a volume 0.1 ml) to remove CD8⁺, CD4⁺ or both CD4⁺ and CD8⁺ T cells, or were injected with anti-asialo-GMI rabbit antibodies to remove NK cells. Twelve days later, when flow cytometry analysis of spleen cells from identically treated mice showed that the targeted T cell populations were depleted, K1735-1D8 cells were transplanted s.c to each T cell-depleted group. K1735-1D8 had similar growth kinetics in mice that had been injected with the anti-CD8 MAb or control rat IgG while removal of CD4⁺ T cells resulted in the growth of K1735-1D8 with the same kinetics as K1735-WT. This failure to inhibit tumor growth after CD4⁺ T cell removal was observed regardless of the presence or absence of CD8⁺ T cells. K1735-1D8 grew in all NK-depleted mice, although more slowly than in the CD4-depleted group. The results are presented in Figure-41C ~~41D~~.

Please replace the paragraph beginning at page 216, line 6, with the following redlined paragraph:

An anti-human CD3 scFv Ig CD80 fusion protein was prepared essentially as described in Examples 1 and 12. The fusion protein comprised an anti-human CD3 scFv fused to wild type IgG1 hinge (SEQ ID NO: 12) and wild type CH2 (SEQ ID NO: 13) and CH3 (SEQ ID NO: 15) domains, fused to CD80 transmembrane and cytoplasmic domains (SEQ ID NO: 29) to

enable cell surface expression of the anti-CD3 scFv. The anti-human CD3 scFv IgG WITH WTCH2CH3--CD80 polynucleotide (SEQ ID NO: 110) encoding the polypeptide (SEQ ID NO: 111) was transfected in Reh cells and into T51 cells (lymphoblastoid cell line). Expression of the anti-human CD3 scFv IgG fusion protein was detected by flow cytometry using FITC conjugated goat anti-human IgG (see methods in Examples 4, 10, 16, 18). The upper graph of Figure 47A illustrates expression of the anti-human CD3 fusion protein on the cell surface of Reh cells, and the lower graph of Figure 47 ~~47B~~ shows expression of the fusion protein on T41 cells.